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Listing of Claims

1. (currently amended) A screening method to obtain a substance with biological activity in the central nervous system (CNS), brain or eye, the method comprising:

administering a test substance to test zebrafish; and

determining effect of the test substance on an activity or function of the central nervous system, brain or eye, or effect of the test substance on a symptom or a disease or disorder of the central nervous system, brain or eye of the test fish,

thereby identifying a substance with said biological activity;

wherein:

(1) the test substance is known to penetrate the blood brain barrier and the method is performed on zebrafish of any age; or

~~or~~ (2) the test substance is known not to penetrate the blood brain barrier and the method

(A) is performed on zebrafish of age less than five days post-fertilisation for molecules of 960 daltons or greater, less than ten days post-fertilization for molecules of less than 960 daltons not effluxed by p-glycoprotein (pgp) or less than eight days post-fertilization for molecules actively effluxed by pgp, and/or

(B) is performed on zebrafish of age at least five days post-fertilisation for molecules of 960 daltons or greater, or at least ten days post-fertilization for molecules of less than 960 daltons not effluxed by pgp and at least eight days post-fertilization for molecules actively effluxed by pgp, and comprises direct delivery of the test substance to bypass the blood brain barrier; or

~~or,~~ (3) without knowing whether or not the test substance is able to penetrate the blood brain barrier, the method is performed on zebrafish of age at least five days post-fertilisation for molecules of 960 daltons or greater, and at least ten days post-fertilization for molecules of less than 960 daltons, with direct delivery of the test substance to bypass the blood brain barrier;

where the stated ages refer to zebrafish grown under standard conditions and are adjusted if the zebrafish are grown under conditions that accelerate or retard achievement of the equivalent stage of development.

2. (original) A method according to claim 1 wherein said direct delivery comprises injection into the brain or eye.

3. (currently amended) A screening method to obtain a substance with biological activity in the central nervous system (CNS), brain or eye, the method comprising:

administering a test substance to test zebrafish fish,

determining effect of the test substance on an activity or function of the central nervous system, brain or eye, or effect of the test substance on a symptom or a disease or disorder of the central nervous system, brain or eye of the test fish,

thereby identifying a substance with said biological activity;

wherein the method (A) is performed for molecules of 960 daltons or greater on zebrafish of age less than five days post-fertilisation and on zebrafish of age at least five days post-fertilisation-, or (B) is performed for molecules of less than 960 daltons on zebrafish of age less than ten days post-fertilisation and on zebrafish of age at least ten days post-fertilization, without direct delivery of the test substance to bypass the blood brain barrier, whereby a substance which has said biological activity and has ability to penetrate the blood brain barrier is obtained;

where the stated ages refer to zebrafish grown under standard conditions and are adjusted if the zebrafish are grown under conditions that accelerate or retard achievement of the equivalent stage of development.

4. (original) A method for obtaining a substance with biological activity in the central nervous system (CNS), brain or eye, the method comprising a screening assay conducted by administering a test substance to test zebrafish whereby a substance with said biological activity is obtained, wherein design of the screening assay employs an algorithm formulated to take into account the presence of and time of formation of a blood brain barrier in the zebrafish.

5. (original) A method according to claim 4 wherein the algorithm is further formulated to take into account whether or not the test substance crosses the blood brain barrier.

6. (cancelled)

7. (currently amended) A method according to ~~any one of claims 1 to 6~~claim 1 further comprising formulating the obtained substance with said biological activity into a composition comprising at least one additional component.

8. (original) A method according to claim 7 wherein the composition comprises a pharmaceutically acceptable vehicle, carrier or excipient.

9. (cancelled)

10. (original) A screening method to obtain a substance with biological activity, which substance does not pass the blood brain barrier, the method comprising:

administering a test substance to test zebrafish

determining effect of the test substance on an activity or function in the zebrafish or effect of the test substance on a symptom or a disease or disorder in the test fish,

thereby identifying a substance with said biological activity,

wherein the method further comprises determining ability or inability of the substance with said biological activity to cross the blood brain barrier in test zebrafish of age at least five days post-fertilisation for molecules of 960 daltons or greater, or at least ten days post-fertilization for molecules of less than 960 daltons, thereby identifying a substance with said biological activity and which does not cross the blood brain barrier;

where the stated ages refer to zebrafish grown under standard conditions and are adjusted if the zebrafish are grown under conditions that accelerate or retard achievement of the equivalent stage of development.

11. (original) A method according to claim 10 comprising identifying the substance with said biological activity in a test zebrafish of age less than five days post-fertilisation for a molecule of 960 daltons or greater, less than ten days post-fertilization for a molecule of less than 960 daltons not effluxed by pgp or less than eight days post-fertilization for a molecule actively effluxed by pgp; where the stated ages refer to zebrafish grown under standard conditions and are adjusted if the zebrafish are grown under conditions that accelerate or retard achievement of the equivalent stage of development.

12. (currently amended) A method according to claim 10 ~~or claim 11~~ further comprising formulating the obtained substance with said biological activity into a composition comprising at least one additional component.

13. (original) A method according to claim 12 wherein the composition comprises a pharmaceutically acceptable vehicle, carrier or excipient.

14. through 17. (cancelled)

18. (currently amended) A method ~~or use~~ according to ~~any one of the preceding claims~~claim 1, further comprising one or more of:
(1) determining whether a test substance is transported by p-glycoprotein;
(2) determining whether a test substance interferes with p-glycoprotein function;
(3) determining whether a test substance alters p-glycoprotein expression;
(4) determining whether a combination of test substances have altered BBB penetration in comparison to a test substance given in isolation;
(5) determining a body: brain drug concentration gradient;
(6) comparing relative efficacies of test substances with desired activity in the central nervous system in the presence of a blood brain barrier;
(7) determining penetration of a test substance across the blood-retinal barrier;
(8) determining whether a test substance is metabolised by the blood brain barrier;
(9) determining whether a test substance is actively transported across the blood brain barrier; or
(10) determining whether a test substance is actively transported into or out of the brain.

19. (cancelled)

20. (cancelled)

21. (currently amended) A method ~~or use~~ according to ~~any one of claims~~claim 18 ~~to 20~~ wherein the test substance is assessed both before and after development of functional p-glycoproteins.

22. (currently amended) A method ~~or use~~ according to claim 21 wherein the test substance is assessed on day 8 or 9 post-fertilisation as well as day 10 or after, where the stated ages refer to zebrafish grown under standard conditions and are adjusted if the zebrafish are grown under conditions that accelerate or retard achievement of the equivalent stage of development.

23. through 40. (cancelled)

41. (new) A method according to claim 3 further comprising formulating the obtained substance with said biological activity into a composition comprising at least one additional component.

42. (new) A method according to claim 4 further comprising formulating the obtained substance with said biological activity into a composition comprising at least one additional component.

43. (new) A method according to claim 3, further comprising one or more of:
(1) determining whether a test substance is transported by p-glycoprotein;
(2) determining whether a test substance interferes with p-glycoprotein function;
(3) determining whether a test substance alters p-glycoprotein expression;
(4) determining whether a combination of test substances have altered BBB penetration in comparison to a test substance given in isolation;
(5) determining a body: brain drug concentration gradient;
(6) comparing relative efficacies of test substances with desired activity in the central nervous system in the presence of a blood brain barrier;
(7) determining penetration of a test substance across the blood-retinal barrier;
(8) determining whether a test substance is metabolised by the blood brain barrier;

(9) determining whether a test substance is actively transported across the blood brain barrier; or
(10) determining whether a test substance is actively transported into or out of the brain.

44. (new) A method according to claim 4, further comprising one or more of:
(1) determining whether a test substance is transported by p-glycoprotein;
(2) determining whether a test substance interferes with p-glycoprotein function;
(3) determining whether a test substance alters p-glycoprotein expression;
(4) determining whether a combination of test substances have altered BBB penetration in comparison to a test substance given in isolation;
(5) determining a body: brain drug concentration gradient;
(6) comparing relative efficacies of test substances with desired activity in the central nervous system in the presence of a blood brain barrier;
(7) determining penetration of a test substance across the blood-retinal barrier;
(8) determining whether a test substance is metabolised by the blood brain barrier;
(9) determining whether a test substance is actively transported across the blood brain barrier; or
(10) determining whether a test substance is actively transported into or out of the brain.

45. (new) A method according to claim 10, further comprising one or more of:
(1) determining whether a test substance is transported by p-glycoprotein;
(2) determining whether a test substance interferes with p-glycoprotein function;
(3) determining whether a test substance alters p-glycoprotein expression;
(4) determining whether a combination of test substances have altered BBB penetration in comparison to a test substance given in isolation;
(5) determining a body: brain drug concentration gradient;
(6) comparing relative efficacies of test substances with desired activity in the central nervous system in the presence of a blood brain barrier;
(7) determining penetration of a test substance across the blood-retinal barrier;
(8) determining whether a test substance is metabolised by the blood brain barrier;
(9) determining whether a test substance is actively transported across the blood brain barrier; or
(10) determining whether a test substance is actively transported into or out of the brain.

46. (new) A method according to claim 11, further comprising one or more of:

(1) determining whether a test substance is transported by p-glycoprotein;

(2) determining whether a test substance interferes with p-glycoprotein function;

(3) determining whether a test substance alters p-glycoprotein expression;

(4) determining whether a combination of test substances have altered BBB penetration in comparison to a test substance given in isolation;

(5) determining a body: brain drug concentration gradient;

(6) comparing relative efficacies of test substances with desired activity in the central nervous system in the presence of a blood brain barrier;

(7) determining penetration of a test substance across the blood-retinal barrier;

(8) determining whether a test substance is metabolised by the blood brain barrier;

(9) determining whether a test substance is actively transported across the blood brain barrier; or

(10) determining whether a test substance is actively transported into or out of the brain.